

# IOWA STATE UNIVERSITY

## Digital Repository

---

Chemistry Publications

Chemistry

---

6-2007

## Cholate–Glutamic Acid Hybrid Foldamer and Its Fluorescent Detection of $\text{Zn}^{2+}$

Zhenqi Zhong  
*Iowa State University*

Yan Zhao  
*Iowa State University, zhaoy@iastate.edu*

Follow this and additional works at: [http://lib.dr.iastate.edu/chem\\_pubs](http://lib.dr.iastate.edu/chem_pubs)

 Part of the [Chemistry Commons](#)

The complete bibliographic information for this item can be found at [http://lib.dr.iastate.edu/chem\\_pubs/164](http://lib.dr.iastate.edu/chem_pubs/164). For information on how to cite this item, please visit <http://lib.dr.iastate.edu/howtocite.html>.

---

This Article is brought to you for free and open access by the Chemistry at Iowa State University Digital Repository. It has been accepted for inclusion in Chemistry Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact [digirep@iastate.edu](mailto:digirep@iastate.edu).

---

# Cholate–Glutamic Acid Hybrid Foldamer and Its Fluorescent Detection of Zn<sup>2+</sup>

## Abstract

A hybrid foldamer containing six cholate units and two glutamic acids was labeled with two pyrenyl groups at the chain ends. Folding was particularly favorable in the presence of zinc(II), as shown by the enhanced emission of the pyrene excimer. The sensitivity of the detection depended on the relative population of the folded and unfolded conformers, being highest when about 90% of the foldamer was in the unfolded state.

## Disciplines

Chemistry

## Comments

Reprinted (adapted) with permission from *Organic Letters* 9 (2007): 2891, doi:[10.1021/ol071130g](https://doi.org/10.1021/ol071130g).  
Copyright 2007 American Chemical Society.

Cholate–Glutamic Acid Hybrid Foldamer  
and Its Fluorescent Detection of  $\text{Zn}^{2+}$ 

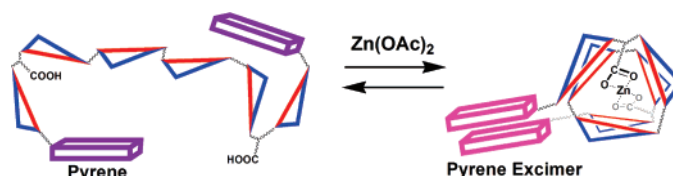
Zhenqi Zhong and Yan Zhao\*

Department of Chemistry, Iowa State University, Ames, Iowa 50011-3111

zhao@iastate.edu

Received May 15, 2007

## ABSTRACT



A hybrid foldamer containing six cholate units and two glutamic acids was labeled with two pyrenyl groups at the chain ends. Folding was particularly favorable in the presence of zinc(II), as shown by the enhanced emission of the pyrene excimer. The sensitivity of the detection depended on the relative population of the folded and unfolded conformers, being highest when about 90% of the foldamer was in the unfolded state.

The conformations of proteins frequently respond to chemical signals, including the presence of metal ions, small molecules, and other biomolecules. This kind of responsiveness can regulate the binding and catalytic properties of proteins<sup>1</sup> and is often the basis of biological signal transduction. Foldamers, as the conformational mimics of biomolecules, have attracted great interest in recent years.<sup>2</sup> Novel molecular sensors and responsive materials can be obtained if the conformation of a foldamer is made to respond to certain chemical species, such as metal ions.<sup>3</sup>

We recently reported cholate foldamers that fold into helical structures *in nonpolar solvents mixed with a small*

*amount of a polar solvent.*<sup>4</sup> Their folding/unfolding is extremely sensitive to solvents. Less than 1% change in solvent composition causes easily detectable changes in the emission of the fluorescent labels on the foldamers.<sup>4a</sup> Because the cholate foldamers are oligoamides, natural amino acids can be readily incorporated to afford desired functional groups. Herein, we report a hybrid foldamer (**1**) whose folding is particularly sensitive to  $\text{Zn}^{2+}$ . Because of the importance of  $\text{Zn}^{2+}$  in biology,<sup>5</sup> development of zinc sensors continues to attract the attention of many chemists.<sup>6,7</sup>

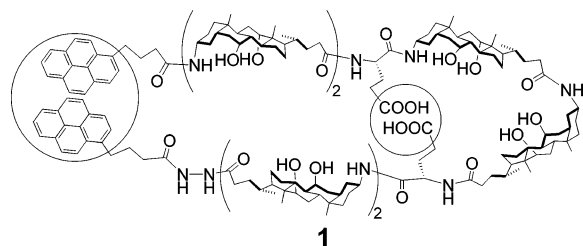
Oligomer **1** was designed so that its folding would bring the two pyrene groups into proximity. To facilitate the excimer formation, we also chose to link the pyrenes to the foldamer by relatively flexible tethers. According to our previous work, three repeat units make up one turn in the oligocholates.<sup>8</sup> Oligomer **1** has six cholates and thus should

(1) (a) Koshland, D. E., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *44*, 98–105. (b) Koshland, D. E., Jr. *Nat. Med.* **1998**, *4*, 1112–1114. (c) Perutz, M. F. *Mechanisms of Cooperativity and Allosteric Regulation in Proteins*; Cambridge University Press: Cambridge, 1990. (d) Hervé, G., Ed. *Allosteric Enzymes*; CRC Press: Boca Raton, Florida, 1989. (e) Kvamme, E.; Pihl, A., Eds. *Regulation of Enzyme Activity and Allosteric Interactions*; Academic Press: New York, 1968.

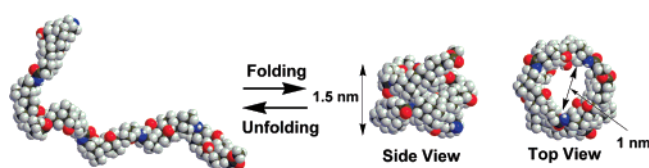
(2) For some representative reviews, see: (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173–180. (b) Kirshenbaum, K.; Zuckermann, R. N.; Dill, K. A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 530–535. (c) Stigers, K. D.; Soth, M. J.; Nowick, J. S. *Curr. Opin. Chem. Biol.* **1999**, *3*, 714–723. (d) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893–4012. (e) Cubberley, M. S.; Iverson, B. L. *Curr. Opin. Chem. Biol.* **2001**, *5*, 650–653. (f) Sanford, A. R.; Gong, B. *Curr. Org. Chem.* **2003**, *7*, 1649–1659. (g) Martinek, T. A.; Fulop, F. *Eur. J. Biochem.* **2003**, *270*, 3657–3666. (h) Cheng, R. P. *Curr. Opin. Struct. Biol.* **2004**, *14*, 512–520. (i) Huc, I. *Eur. J. Org. Chem.* **2004**, 17–29. (j) Licini, G.; Prins, L. J.; Scrimin, P. *Eur. J. Org. Chem.* **2005**, 969–977.

(3) For several examples of metal-induced folding or unfolding, see: (a) Prince, R. B.; Okada, T.; Moore, J. S. *Angew. Chem., Int. Ed.* **1999**, *38*, 233–236. (b) Barboiu, M.; Lehn, J.-M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5201–5206. (c) Kolomiets, E.; Berl, V.; Odriozola, I.; Stadler, A.-M.; Kyritsakas, N.; Lehn, J.-M. *Chem. Commun.* **2003**, 2868–2869. (d) Zhang, F.; Bai, S.; Yap, G. P. A.; Tarwade, V.; Fox, J. M. *J. Am. Chem. Soc.* **2005**, *127*, 10590–10599 and references therein. (e) Nicoll, A. J.; Miller, D. J.; Fuetterer, K.; Ravelli, R.; Allemann, R. K. *J. Am. Chem. Soc.* **2006**, *128*, 9187–9193.

(4) (a) Zhao, Y.; Zhong, Z. *J. Am. Chem. Soc.* **2005**, *127*, 17894–17901. (b) Zhao, Y.; Zhong, Z. *J. Am. Chem. Soc.* **2006**, *128*, 9988–9989. (c) Zhao, Y.; Zhong, Z. *Org. Lett.* **2006**, *8*, 4715–4717. (d) Zhao, Y.; Zhong, Z.; Ryu, E.-H. *J. Am. Chem. Soc.* **2007**, *129*, 218–225.



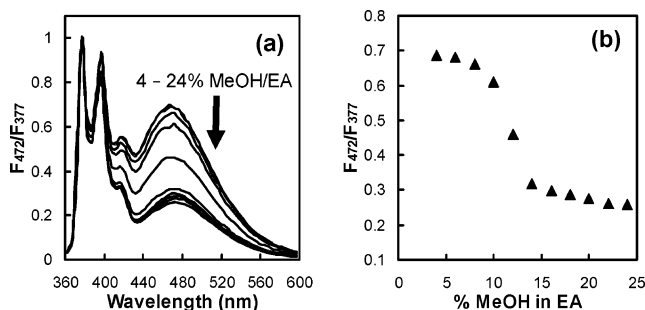
make two full turns, assuming that the amino acids do not cause significant perturbation to the periodicity of the foldamer.<sup>9</sup> The end-to-end distance, according to the CPK model of a folded cholate hexamer, is 1–1.5 nm but can reach several nanometers for the unfolded conformer (Figure 1). Therefore, the pyrene excimer should be easily formed



**Figure 1.** Molecular models of an unfolded and folded cholate hexamer.

in the folded state but largely absent in the unfolded state.

The pyrene excimer indeed is a good indicator for the folding.<sup>10</sup> As shown by Figures 2a and 2b, when the fluorescence of hexamer **1** is examined in mixtures of methanol and ethyl acetate (EA), the excimer/monomer ratio



**Figure 2.** (a) Normalized fluorescence spectra of oligomer **1** in different mixtures of methanol and EA. (b) The excimer/monomer ratio ( $F_{472}/F_{377}$ , the emission intensity at 472 nm over that at 377 nm) as a function of solvent composition.

(i.e.,  $F_{472}/F_{377}$ ) decreases from  $\sim 0.7$  in 4–8% MeOH to  $\sim 0.3$  in  $>14\%$  MeOH. This change corresponds to a transition from a compact, folded conformation to an extended, unfolded one. The conformational change is highly cooperative, with most of the change taking place within a narrow range of solvent change.

The data in Figure 2b are fitted to a two-state transition model (eq 1), from which the folding/unfolding equilibrium at every solvent composition can be determined.<sup>11,12</sup> As shown by Figure 3a,  $>90\%$  of the oligomer is folded in 4–8% MeOH/EA. Addition of another few percent of methanol quickly unfolds the oligomer. With  $>14\%$  MeOH, over 90% of oligomer **1** becomes unfolded.



Compared to the parent foldamer with no  $\alpha$ -amino acids in the backbone, oligomer **1** folds much better. For example, the parent cholate hexamer does not fold in MeOH/EA mixtures but folds only when hexane is added to the mixture.<sup>13</sup> Even in hexane/EA (2:1), a nonpolar mixture proving the best for the folding of the oligocholates, the parent cholate hexamer is folded only with  $<5\%$  MeOH.<sup>4a,d</sup> Previously, the incorporation of two methionines into the cholate hexamer was found to help folding slightly,<sup>4b</sup> possibly

(5) For some recent reviews, see: (a) Lim, N. C.; Freake, H. C.; Brueckner, C. *Chem.-Eur. J.* **2005**, *11*, 38–49. (b) Thompson, R. B. *Curr. Opin. Chem. Biol.* **2005**, *9*, 526–32. (c) Kikuchi, K.; Komatsu, K.; Nagano, T. *Curr. Opin. Chem. Biol.* **2004**, *8*, 182–191. (d) Jiang, P.; Guo, Z. *Coord. Chem. Rev.* **2004**, *248*, 205–229.

(6) For some recent examples of fluorescent  $\text{Zn}^{2+}$  sensors, see: (a) Kimura, E.; Aoki, S.; Kikuta, E.; Koike, T. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3731–3736. (b) Hanaoka, K.; Kikuchi, K.; Kojima, H.; Urano, Y.; Nagano, T. *Angew. Chem., Int. Ed.* **2003**, *42*, 2996–2999. (c) Taki, M.; Wolford, J. L.; O'Halloran, T. V. *J. Am. Chem. Soc.* **2004**, *126*, 712–713. (d) Komatsu, K.; Kikuchi, K.; Kojima, H.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2005**, *127*, 10197–10204. (e) Royzen, M.; Durandin, A.; Young, V. G.; Geacintov, N. E.; Canary, J. W. *J. Am. Chem. Soc.* **2006**, *128*, 3854–3855. (f) Liu, Y.; Zhang, N.; Chen, Y.; Wang, L.-H. *Org. Lett.* **2007**, *9*, 315–318. (g) Nolan, E. M.; Ryu, J. W.; Jaworski, J.; Feazell, R. P.; Sheng, M.; Lippard, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 15517–15528. (h) Van D. Elisabeth M. W. M.; Evers, T. H.; Dekkers, L. M.; Meijer, E. W.; Klomp, L. W. J.; Merkx, M. *J. Am. Chem. Soc.* **2007**, *129*, 3494–3495.

(7) For some earlier examples of fluorescent  $\text{Zn}^{2+}$  sensors, see: (a) Bird, A. J.; Turner-Cavet, J. S.; Lakey, J. H.; Robinson, N. J. *J. Biol. Chem.* **1998**, *273*, 21246–21252. (b) Goodall, W.; Williams, J. A. G. *Chem. Commun.* **2001**, 2514–2515. (c) Prodi, L.; Montalti, M.; Bradshaw, J. S.; Izatt, R. M.; Savage, P. B. *J. Inclusion Phenom. Macro. Chem.* **2001**, *41*, 123–127. (d) Rurack, K. *Spectrochim. Acta A* **2001**, *57*, 2161–2195. (e) Gee, K. R.; Zhou, Z.-L.; Qian, W.-J.; Kennedy, R. J. *J. Am. Chem. Soc.* **2002**, *124*, 776–778. (f) Jiang, P.; Chen, L.; Lin, J.; Liu, Q.; Ding, J.; Gao, X.; Guo, Z. *Chem. Commun.* **2002**, 1424–1425. (g) Yang, R.-H.; Li, K.-A.; Wang, K.-M.; Zhao, F.-L.; Li, N.; Liu, F. *Anal. Chem.* **2003**, *75*, 612–621.

(8) A similar preference for the trimeric periodicity was discovered by Sanders and co-workers, who reported trimeric cyclic cholate esters were more stable than other cyclic structures under thermodynamic control. For details, see: Brady, P. A.; Bonar-Law, R. P.; Rowan, S. J.; Suckling, C. J.; Sanders, J. K. M. *Chem. Commun.* **1996**, 319–320.

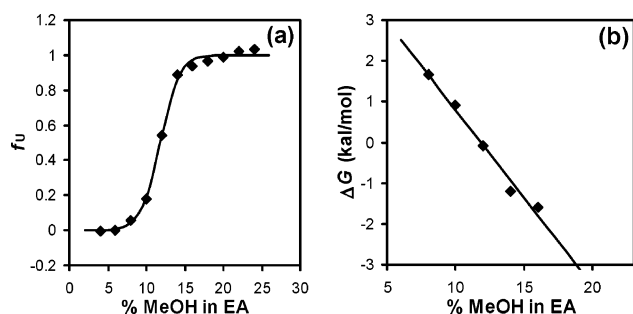
(9) The contribution of two  $\alpha$ -amino acids to the length of a cholate hexamer is insignificant because of the much larger size of a cholate unit.

(10) We also attempted to monitor the folding by CD spectroscopy, but no CD signals were detected in the pyrene absorption region. Amide absorption cannot be studied because it overlaps with that of ethyl acetate.

(11) The two-state model works well with foldamers with relatively rigid repeating units. See: Prince, R. B.; Saven, J. G.; Wolynes, P. G.; Moore, J. S. *J. Am. Chem. Soc.* **1999**, *121*, 3114–3121 and references therein.

(12) For detailed procedures on how to obtain folding energies from solvent-titration curves, see: (a) Pace, C. N. In *Methods in Enzymology*; Hirs, C. H. W., Timasheff, S. N., Eds.; Academic Press: New York, 1986; Vol. 131, pp 266–280. (b) Pace, C. N.; Shirley, B. A.; Thomson, J. A. In *Protein Structure: A Practical Approach*; Creighton, T. E., Ed.; IRL Press: New York, 1989; pp 311–330.

(13) The folded oligocholate has a hydrophobic exterior and a hydrophilic interior. A small amount of the polar solvent is microphase separated from the bulk to the interior. Phase separation of methanol is easier in EA/hexane than in EA because methanol is completely miscible with EA but nearly immiscible with hexane. For detailed discussions, see ref 4a and 4d.

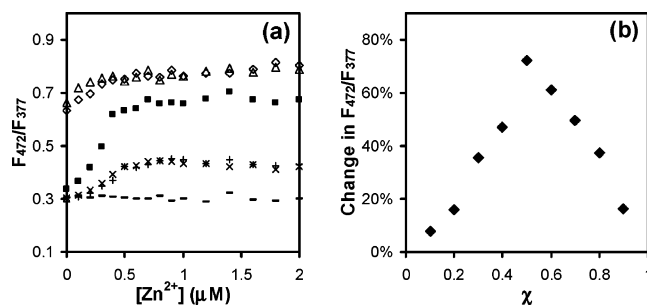


**Figure 3.** (a) Fraction of the unfolded conformer in oligomer **1** as a function of the volume percentage of MeOH in EA. The theoretical curve is nonlinear least-squares fitting to a two-state transition model. (b) Unfolding free energies for oligomer **1** as a function of solvent composition (see the Supporting Information for details).

because the amino acids introduced some degree of flexibility into the backbone.<sup>14</sup> Since the difference between methionine and glutamic acid lies in the side chain, the much better folding in **1** must come from the carboxyl groups, likely due to some kind of intramolecular hydrogen-bonding interactions.

With a highly cooperative folding/unfolding transition, oligomer **1** should be very useful as a sensor. For example, a divalent metal ion should cross-link the two carboxylic acids and stabilize the folded state. Indeed, a screening reveals that several divalent cations promote the folding, and zinc(II) turns out particularly effective.

Figure 4a shows the pyrene excimer/monomer ratio as a



**Figure 4.** (a) Excimer/monomer ratio ( $F_{472}/F_{377}$ ) as a function of  $[\text{Zn}(\text{OAc})_2]$  in 5% ( $\Delta$ ), 10% ( $\diamond$ ), 15% ( $\blacksquare$ ), 20% ( $\times$ ), 25% ( $+$ ), and 100% ( $-$ ) MeOH in EA.  $[\text{I}] = 0.5 \mu\text{M}$ . (b) The Job plot for the binding between foldamer **1** and  $\text{Zn}(\text{OAc})_2$  in 15% MeOH/EtOAc. The total concentration of **1** and  $\text{Zn}(\text{OAc})_2$  was kept at  $1.0 \mu\text{M}$ .  $\chi = [\text{Zn}(\text{OAc})_2]/([\text{I}] + [\text{Zn}(\text{OAc})_2])$ .

function of  $\text{Zn}^{2+}$  concentration in six different MeOH/EA mixtures. In 5 and 10% MeOH, addition of  $\text{Zn}^{2+}$  only

(14) A certain level of flexibility is beneficial because the folded state will not be overly strained; too much flexibility, however, is detrimental to the folded conformer because the loss of entropy will be too large during folding.

increases the excimer/monomer ratio slightly (Figure 4a,  $\Delta$  and  $\diamond$ ). This is reasonable because oligomer **1** is largely folded in these solvents according to Figures 2b and 3a. Complexation with  $\text{Zn}^{2+}$  probably tightens the structure slightly, resulting in a small enhancement of the excimer.

The most significant enhancement of the pyrene excimer by  $\text{Zn}^{2+}$  happens in 15% MeOH (Figure 4a,  $\blacksquare$ ). The binding must be extremely strong, as shown by the sharp transition of the  $F_{472}/F_{377}$  curve at  $0.5 \mu\text{M}$  of zinc ions, or at a 1:1 ratio. To further confirm the binding stoichiometry, the Job analysis is carried out, in which the total concentration of foldamer **1** and  $\text{Zn}^{2+}$  is kept at  $1.0 \mu\text{M}$  while the ratio between the two is varied from 1:9 to 9:1. The largest change in  $F_{472}/F_{377}$  clearly occurs at  $\chi = 0.5$  (Figure 4b), consistent with a 1:1 complex being formed during titration. A nonlinear least-squares fitting of data in Figure 4a shows the binding constant to be at least  $10^8 \text{ M}^{-1}$ .

The binding between **1** and  $\text{Zn}^{2+}$  is still detectable in 20 and 25% MeOH (Figure 4a,  $\times$  and  $+$ , respectively), but the enhancement in  $F_{472}/F_{377}$  is less significant than that in 15% MeOH. Note that in 15, 20, and 25% MeOH/EA, even after **1** is saturated with  $\text{Zn}^{2+}$ , the excimer/monomer ratio never reaches the value for the fully folded, uncomplexed foldamer (i.e., ca. 0.7 according to Figure 2b). In our hands, most metal ions including  $\text{Zn}^{2+}$  quench the fluorescence of both the pyrene monomer and the excimer (vide infra).<sup>15</sup> Quenching by the bound  $\text{Zn}^{2+}$ , however, cannot explain the lower  $F_{472}/F_{377}$  value in 15–25% MeOH, because addition of  $\text{Zn}^{2+}$  in 5 and 10% MeOH actually increases  $F_{472}/F_{377}$  slightly. When an amphiphilic oligomer such as **1** folds in a mixture of methanol and EA, there is considerable change in local solvent composition. This change is determined both by the foldamer<sup>4a,4d,13</sup> and by the overall solvent composition.<sup>16</sup> Even though the same  $\text{Zn}^{2+}$ -complexed foldamer is involved in all the titration studies, the local solvent composition is actually different in each case because the percentage of MeOH in the bulk is different. In other words, the pyrene excimer formed during the  $\text{Zn}^{2+}$  titration in different solvents has different solvent shells, which are possibly responsible for the difference in  $F_{472}/F_{377}$  at the plateau regions in Figure 3a.<sup>17,18</sup>

Zinc ions are no longer able to promote the pyrene excimer in neat methanol (Figure 4a,  $-$ ). Given the 1.4 nm heat-to-

(15) We find it difficult to fit the quenching data directly to a 1:1 binding isotherm. The apparent binding constants are quite different depending on the wavelength at which the quenching is monitored. This is probably due to multiple quenching mechanisms being involved. The bound metal ion, the formation of the pyrene excimer, and the change in local solvent composition during folding all affect the emission of both the monomer and the excimer, most likely to different extents.

(16) Ryu, E.-H.; Jie, Y.; Zhong, Z.; Zhao, Y. *J. Org. Chem.* **2006**, *71*, 7205–7213.

(17) Previously, the change in local solvent composition during folding was found to affect the fluorescence of an NBD label on the foldamer significantly. Such a solvent effect was completely absent if the fluorophore was attached to the cholate monomer, which was incapable of folding. Thus, this solvent effect differs from a generic solvent effect on a polarity-sensitive fluorophore. For details, see ref 4a.

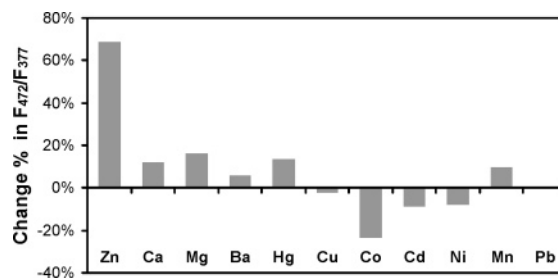
(18) Other factors may also contribute to the different  $F_{472}/F_{377}$  values for the zinc-complexed foldamer in different solvents. For example, the (zinc-complexed) folded and the (zinc-free) unfolded foldamers have different hydrodynamic radii. A change in the solvent composition changes the viscosity of the solvent system, which also may affect the emission of a fluorophore.



tail length of a single cholate unit, oligomer **1** is unlikely to bind  $\text{Zn}^{2+}$  in the unfolded state, especially if a 1:1 complex is involved. When the folded conformer becomes disfavored by the solvent, binding has to overcome the unfavorable folding reaction and becomes weaker.<sup>4b,4d</sup> The microphase separation of solvents that helps the folding of the oligocholate is not possible in MeOH, a single-solvent system.<sup>19</sup> Also, methanol itself represents a poor medium for a folded oligocholate, which has a hydrophobic exterior. Poor binding of  $\text{Zn}^{2+}$  in 100% methanol, therefore, is fully expected.

Last, we studied the effects of other divalent cations on the folding of oligomer **1**, including  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Mn}^{2+}$ , and  $\text{Pb}^{2+}$ , as well as some monovalent cations, such as  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{NH}_4^+$ . Some of them (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Ba}^{2+}$ ) also promote the excimer formation.<sup>20</sup> Their binding is generally weaker than that of  $\text{Zn}^{2+}$ . The monovalent cations show no effect at all toward the excimer/monomer ratio.<sup>21</sup> The rest of the divalent cations all behave differently, sometimes giving an overall decrease in  $F_{472}/F_{377}$  throughout the titration (e.g.,  $\text{Co}^{2+}$ )<sup>22</sup> and other times showing an increase initially followed by a decrease (e.g.,  $\text{Mn}^{2+}$ ).<sup>23</sup> It seems that the monomer and the excimer of pyrene are quenched to different degrees by different metal ions. Figure 5 compares these metal ions in a bar graph. The excimer/monomer ratio responds quite differently to the same concentration ( $0.4 \mu\text{M}$ ) of divalent cations, but  $\text{Zn}^{2+}$  clearly stands out by its strong ability to enhance the excimer formation.

In summary, we have designed a foldamer that uses the formation of a pyrene excimer as a signature for its folding and two internal carboxylic acids to chelate with a divalent metal ion. Its specificity for  $\text{Zn}^{2+}$  mostly results from other



**Figure 5.** Change in the excimer/monomer ratio of foldamer **1** ( $0.5 \mu\text{M}$ ) in 15% MeOH/EtOAc upon addition of various divalent metal ions ( $0.4 \mu\text{M}$ ).

divalent metal ions that bind with the foldamer less strongly and/or interact with the pyrene labels in different fashions. Because the foldamer senses the metal ion by going from the unfolded to the folded conformation (shown by the change in the  $F_{472}/F_{377}$  value), the most sensitive detection of  $\text{Zn}^{2+}$  happens in 15% MeOH/EA. Little change in  $F_{472}/F_{377}$  occurs in solvents with lower methanol, as the foldamer is already folded without the metal ion. Higher methanol in the solvent mixture destabilizes the folded conformer, weakening the binding to the metal ion as a result.<sup>4b</sup>

**Acknowledgment.** Acknowledgment is made to the Roy J. Carver Charitable Trust and the Iowa State University Research Foundation for the support of this research. We thank Dr. Basudeb Saha at Iowa State University for the use of the spectroscopy facility.

**Supporting Information Available:** Experimental details, two-state analysis, and fluorescence data (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL071130G

(19) Folding is made possible by both the curvature of the cholate (toward the hydrophilic face) and the entrapped polar solvents (as a result of microphase separation), which simultaneously solvate the multiple inward-facing NH/OH groups on the hydrophilic faces of the cholates.

(20) Figures 2S, 3S, and 4S in the Supporting Information.

(21) Figures 5S, 6S, and 7S in the Supporting Information.

(22) Figure 8S in the Supporting Information.

(23) Figure 9S in the Supporting Information.